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Effect of the CO_2 Milliwatt Laser on Neuroma Formation in Rats

Rafic Kuzbari, MD, Christian Liegl, MD, Christoph Neumayer, Hermann Moser, Georg Burggasser, MD, Jürgen Holle, MD, Helmut Gruber, MD, and Wolfgang Happak, MD

Department of Plastic Surgery, Wilhelminen Hospital, A-1171 Vienna, Austria (R.K., J.H.); Department of Traumatology (C.L.), Third Department of Anatomy (C.N., H.M., G.B., H.G.), and Department of Plastic Surgery (W.H.), University of Vienna, A-1090 Vienna, Austria

Background and Objective: The purpose of this study was to determine whether the milliwatt laser can suppress neuroma formation at the end of a divided nerve.
Study Design/Materials and Methods: The peripheral nerves of eight rats were transected with microscissors and the cross-sectional area of their proximal ends was irradiated using the CO_2 milliwatt laser. The power ranges used were similar to those applied to weld neural tissue.
Results: None of the eight irradiated nerve ends formed a neuromatous bulb and only one of them regenerated into the surrounding tissues. Histologically, these nerve ends did not show the disorganized picture of classic neuromas. On morphometric measurements, they contained less connective tissue than the control nerve ends ($P < 0.001$) and their nerve fibers were larger in diameter ($P < 0.001$) and better myelinated ($P < 0.001$).
Conclusion: These findings in rats show that the CO_2 milliwatt laser has the ability to suppress neuroma formation at the end of a divided nerve.

Key words: CO_2 laser, histology, neuroma prevention, peripheral nerve, traumatic neuroma

INTRODUCTION

Neuromas arise in a divided nerve when the axons of the proximal end regenerate in a disorganized fashion into the surrounding connective tissue [1]. Despite the many operative techniques that have been proposed to date, no single method reliably prevents the formation of a painful neuroma and its potentially devastating sequelae [1–3]. A method that consistently prevents neuroma formation must successfully seal severed fasciculi in order to hinder the escape of regenerating axons into the surrounding tissue [4]. The CO_2 laser in the milliwatt power range is known to have the ability to weld and seal connective tissue [5]. It has therefore been used in the repair of peripheral nerves to weld the epineurial sheath and to confine the growth of the regenerating axons within the sheath boundaries [6–9]. However, it is not yet known whether the milliwatt laser has the ability to suppress neuroma formation at the end of a divided nerve. To answer this question, we applied CO_2 laser light in the milliwatt range to the proximal end of divided tibial and peroneal nerves in rats and assessed its effect on neuroma formation.

MATERIALS AND METHODS

Surgical Procedures

Eight adult Sprague-Dawley rats, weighing 300–400 g, were each anesthetized by injecting pentobarbital (40 mg/kg) into the peritoneum.

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Address reprint requests to Dr. Rafic Kuzbari at Schreiberweg 47, A-1190 Vienna, Austria.

The right tibial and peroneal nerves were then exposed by an incision in the dorsal aspect of the thigh. Using microscissors, both nerves were transected at the same level, distal to their origin from the sciatic nerve. One cm length of the distal nerve ends was resected to prevent axonal regeneration into them.

Laser irradiation was performed using a low-energy CO_2 Laser (Heraeus Corp., model Heracure LS 500, Hanau, Germany) mounted to an operating microscope (Zeiss, OPMI-MD, Germany). The focused laser beam was delivered in continuous mode at 80 mW with a spot size of 135 μm and at a focal length of 250 mm. Laser energy was evenly distributed on the entire cross-sectional area of the proximal nerve end until it created discreet blanching of the neural tissue without any carbonization. The total irradiation time needed for the cross-sectional area of one nerve was ~20 seconds. The treatment energy was 1,600 J and the power density resulting at tissue level was 480 W/cm^2.

In four of the eight rats, the proximal end of the peroneal nerve was irradiated and in the four other rats, the proximal end of the tibial nerve was irradiated. The ipsilateral proximal ends of the tibial, respectively, peroneal nerves were left untreated after transection and served as controls. In this way, both the laser-irradiated and the control nerves were placed in the same wound and were subjected to the same microenvironmental stimuli for regeneration.

To differentiate the tibial from the peroneal nerve and to be able to identify the level of nerve transection at the time of the second operation, the proximal end of the tibial nerve was marked with a single 10-0 nylon suture placed into the epifascicular epineurium. As it was transected at the same level, the peroneal nerve end was left unmarked. The rats were then returned to their cages without immobilization of their operated hind legs.

After 12 weeks the rats were reanesthetized and the tibial and peroneal nerves were reexplored and separated using the operating microscope. The previously placed marking suture was used to identify the level at which both proximal nerve ends were transected. Macroscopical evidence of neuroma formation was recorded (i.e., a neuromatous bulb or disorganized growth into the surrounding tissue). The nerves were then removed and carefully marked in a proximal to distal orientation. The animals were thereafter sacrificed by an overdose of anesthetic solution.

Histological Analysis

The nerve segments were immediately processed for histological and quantitative morphometric analysis. To maintain their length, the segments were stretched on a polystyrene board. They were then fixed in a solution of 3% glutaraldehyde buffered with cacodylate, postfixed with 1% osmium tetroxide, contrasted with uranyl acetate, and embedded in epoxy resin. Serial, 1-μm-thick sections were cut transversely at 0.5–1.5 mm intervals, at the same level along the length of all specimens. These sections were stained with toluidine blue and fuchsin and examined by light microscopy. Other 50-nm-thick sections were cut with an ultramicrotome (Ultracut E, Reichert-Jung, Germany) and examined by electron microscopy (Zeiss, EM 9, Germany).

Morphometric measurements of all sections were carried out at × 3,680 magnification using a digital pen linked to a semiautomatic particle analysing computer (Lucia, Laboratory Imaging, Czesh Republic) [10]. The neural tissue-to-connective tissue ratio was calculated by measuring the area occupied by the fascicles in one third of the cross-sectional area. This ratio was expressed as the percentage of the neural tissue present in the entire cross section. In addition the particle analyzing computer was used to determine the average diameter, the average amount of myelination, and the average number of the nerve fibers.

Statistical Analysis

The computer software package Sigmastat (Jandel Scientific Corp., San Francisco, CA) was used for statistical analysis. Data are summarized as median and interquartile range (range between the 25th and 75th percentile). To determine if there were significant differences in the morphometric measurements of the laser-irradiated and the control nerves we used the Man-Whitney rank sum test. Differences were considered significant at $P < 0.05$.

RESULTS

Gross Morphology

With one exception, the laser-irradiated nerves showed no macroscopical evidence of neuroma formation. None of the eight irradiated nerve ends formed a neuromatous bulb, and only one of them tended to regenerate into the surrounding tissues. Three of the eight control nerves formed well-defined neuromatous bulbs at

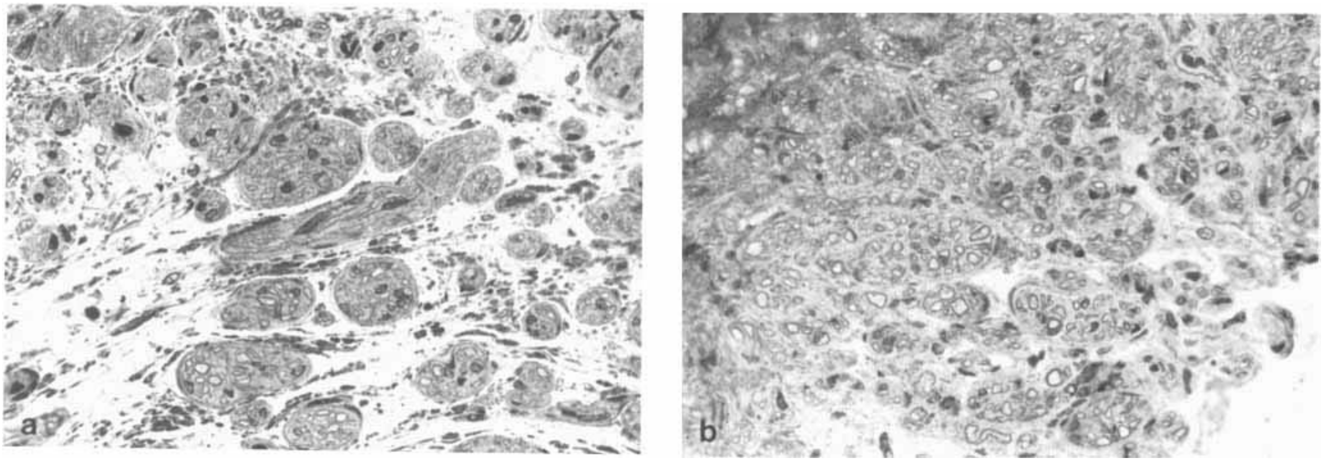

Fig. 1. Transverse section of the proximal nerve ends (toluidine blue and fuchsin, × 50). (**a**) In the control group, connective tissue is preponderent; small nerve fibers are identified in cross section and in longitudinal orientation. (**b**) In the laser-irradiated group, the nerve fibers are grouped into densely packed minifascicles, which are oriented in the same direction.

their proximal ends, and five of them did regenerate and extensively attach to the surrounding tissues.

Histology

Light microscopic examination of the control nerves distal to the marking suture showed the features of a classic neuroma [1,4] (Fig. 1a). Connective tissue with many capillaries was preponderant and a disorganized tangle of small nerve fibers interlaced and crossed through the tissue without any orientation. Light microscopic examination of the laser-irradiated nerves distal to the marking suture showed the picture of a more organized nerve regeneration (Fig. 1b). Less connective tissue was found and the regenerating nerve fibers were grouped into small, densely packed minifascicles. These minifascicles were surrounded by perineurium and were mostly oriented in the same direction.

On electron microscopy of the ultrathin sections, no myofibroblasts were found either in the laser-irradiated nor in the control nerve ends. Myelinated and unmyelinated axons, surrounded by perineurial cells and collagen fibers, were seen in both nerve ends (Fig. 2a,b).

The morphometric measurement of the neural tissue-to-connective ratio showed a significantly higher percentage of neural tissue in the cross-sections of the laser-irradiated nerve ends, in comparison to the cross-sections of the control nerve ends ($P < 0.001$) (Table 1). The microscopic impression was thus confirmed that less connective and more neural tissue are present in the ends of the laser-irradiated nerves. In addition, morphometric analysis of the neural tissue showed that the fibers of the irradiated nerve ends were larger in diameter ($P < 0.001$) and better myelinated ($P < 0.001$) (Table 1). There were no statistically significant differences between the morphometric measurements of the tibial and the peroneal nerves.

DISCUSSION

This study in rats shows that the CO_2 milliwatt laser has the ability to suppress neuroma formation at the end of a divided nerve. Macroscopically, none of the laser-irradiated nerve ends formed a neuromatous bulb and only one did regenerate into the surrounding tissues. Histologically, the laser-irradiated nerve ends did not show the usual picture of a classic neuroma. They contained significantly less connective tissue than the control neuromas. Their regenerating nerve fibers were grouped into densely packed minifascicles, which were mostly oriented in the same direction, unlike the convoluted and disorganized appearance of classic neuromas [1,4] (Fig. 1a,b). In addition, these nerve fibers were significantly better myelinated and larger in diameter than the immature fibers of the control neuromas (Table 1). Unmyelinated and small-diameter nerve fibers are a typical finding in classic neuromas [4,11]. Myofibroblasts, which have been linked with neuroma pain in humans [12], were not

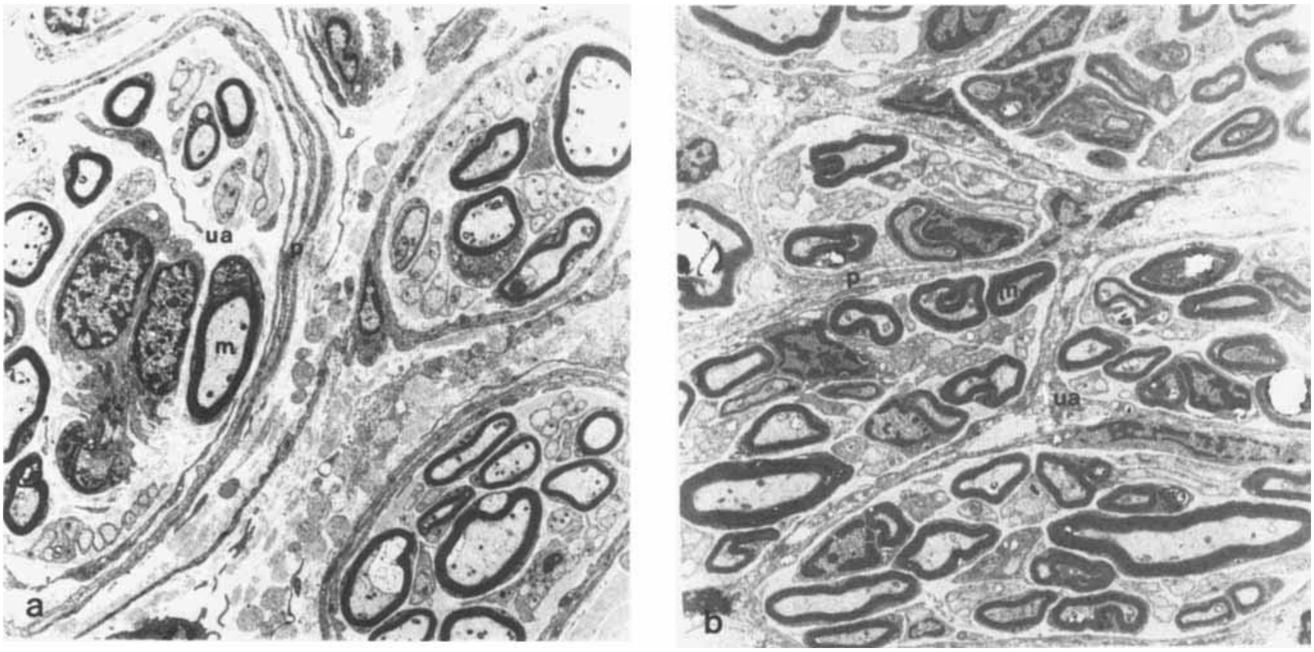

Fig. 2. Electron microscopy of the proximal nerve ends (uranyl acetate, × 7500). The ultrastructure is similar (**a**) in the control (**b**) and in the laser-irradiated groups. m = myelinated axons, ua = unmyelinated axons, p = perineurial cells. Note that the nerve fibers are more densely packed in the laser-irradiated nerve ends.

TABLE 1. Morphometric Measurements of the Proximal Nerve Ends[†]

	Laser-irradiated	Control
Percentage of neural tissue	50.20*	28.55
Average diameter of a nerve fiber (μ)	1.16 (1.92)*	1.04 (1.69)
Average thickness of the myelin sheath of a nerve fiber (μ)	0.86 (0.47)*	0.84 (0.40)
Number of nerve fibers/mm^2	24,170 (17,600)	21,720 (5,960)

[†]Values are median (interquartile range).
*Statistically significant vs. the "control" value ($P < 0.001$)

found either in the irradiated nor in the control nerve ends.

Other studies that attempted to suppress neuroma formation by using lasers have yielded controversial results [13–15]. In these studies, the laser was used at high power ranges as an instrument to transect the peripheral nerve. In our study, however, the nerve was transected with microscissors and the CO_2 milliwatt laser was used at low power ranges to irradiate the cross-sectional area of the proximal nerve end. These power ranges were similar to those applied to weld and seal neural tissue [7–9]. The welding mechanism has been thereby characterized as a thermal effect that causes uncoiling of collagen followed by random reformation of inter- and intrastrand crosslinks upon cooling [16].

This thermal effect is possibly the mechanism by which the CO_2 laser did suppress neuroma formation in our study. For many years the proximal ends of transected nerves have been heated by electrocoagulation to prevent neuroma formation clinically [17,18]. The unpredictable results of this coagulation technique were recently improved by a lower energy electrocoagulation method [19] that reduces damage to the neural tissue. However, to heat up a nerve end and at the same time keep tissue damage to a minimum, the milliwatt laser, which allows the precise application of small amounts of energy, seems to be a more appropriate tool than the electrocautery.

The uncontrolled amount of thermal energy applied to the neural tissue may explain some of the contradictory results found by previous investigators who used the laser at higher power ranges to transect the nerve [13–15]. In our study, an uneven distribution of this thermal energy on the entire cross-sectional area of the nerve end may also explain why laser irradiation failed to suppress regeneration into the sur-

rounding tissues in one of the eight irradiated nerves.

In conclusion, despite the small number of animals it includes, this study shows that the CO_2 milliwatt laser can successfully suppress neuroma formation in the rat, which is known for its superlative capacity of nerve regeneration [7]. Clinical implications remain, however, speculative at the present time.

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